

Responses of cardiac tissue to simulated weightlessness

Candice Tahimic^{1,2}, Sonette Steczina^{1,3}, Masahiro Terada¹, Yasaman Shirazi-Fard^{1,2}, Ann-Sofie Schreurs^{1,4}, David Goukassian⁵, Ruth K. Globus¹

¹Space Biosciences Division, NASA Ames Research Center, ²Wyle Laboratories, ³Blue Marble Sciences, ⁴NASA Academic Mission Services, ⁵Temple University

INTRODUCTION

Risk of adverse cardiovascular changes during and after spaceflight

- Space travel causes both rapid and delayed effects on the cardiovascular system.
- Exposure to microgravity causes a cephalad shift of body fluids leading to increased heart stroke volume and a rise in left ventricular end diastolic dimensions. Fluid shifts can diminish the ability of the heart to cope with gravitational stress immediately after returning to earth, leading to orthostatic intolerance (syncope) observed in some astronauts.
- Earth-based models of prolonged exposure to microgravity (long-duration hindlimb unloading in mice) causes cardiac enlargement, depressed contractility, and increased susceptibility to arrhythmias.

Spaceflight effects on oxidative stress-related genes in the heart

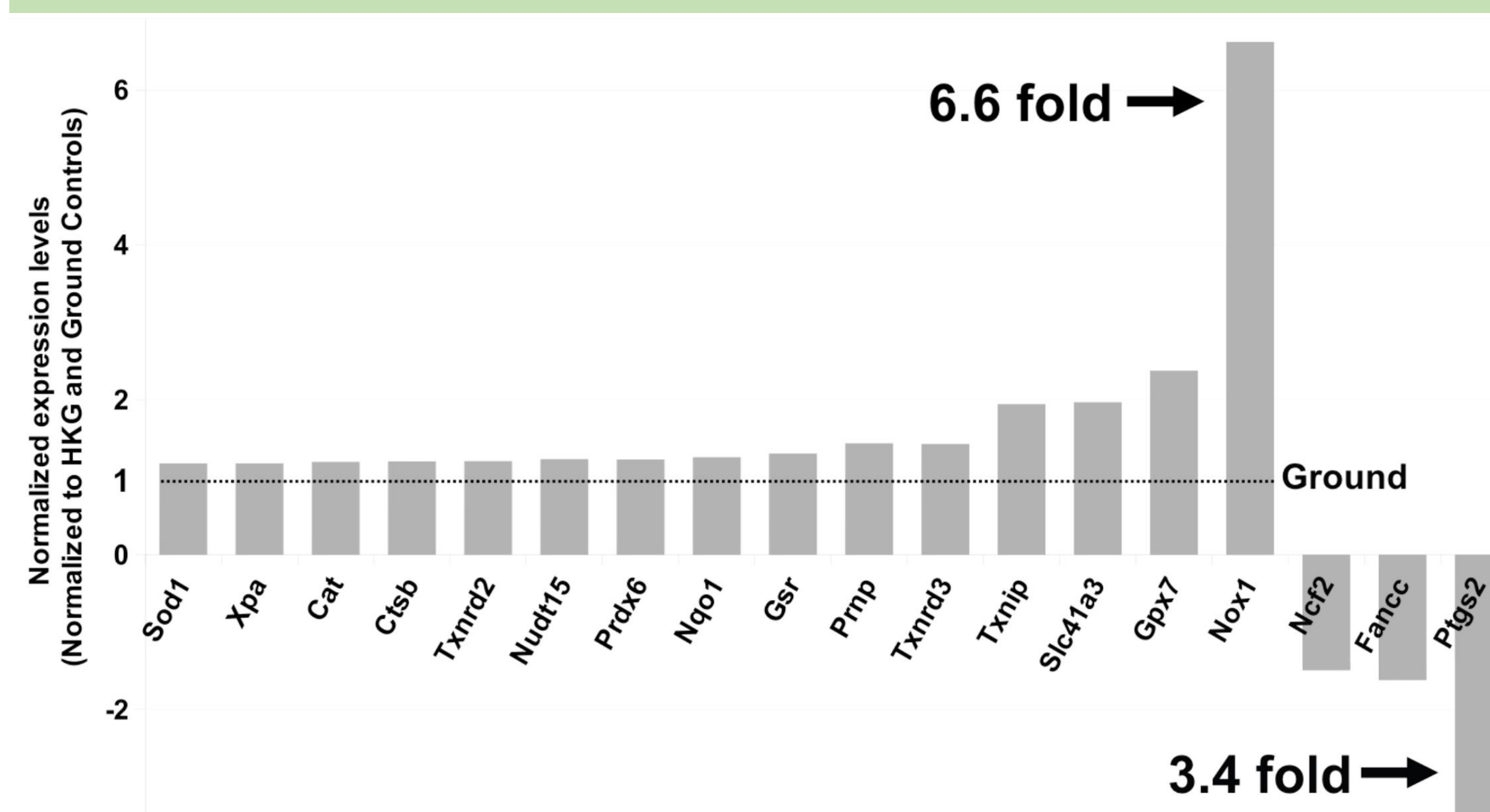


Figure 1. Spaceflight induces expression or redox-related genes. Hearts from rodents flown in STS-131 were examined for expression of genes regulating redox balance using a real time PCR array of 84 genes related to oxidative metabolism. Of the 84 genes analyzed, 15 genes were up-regulated and three were down-regulated in flight samples ($p < 0.05$).

PURPOSE OF THE STUDY

Determine the time course and persistence of simulated weightlessness-induced changes in the heart, as well as the contributions of sex and age to the observed responses.

HYPOTHESES

Long duration simulated weightlessness and subsequent recovery causes select and persistent changes in gene expression and oxidative defense-related pathways.

EXPERIMENT PLAN

Groups and treatments

- *Fisher rats*: Three month old (Young) males and females; nine month old (Aged) males
- *Treatment*: Hindlimb unloading (HU) for 7, 14, 21 and 90 days with corresponding ambulatory (no HU) controls; or HU followed by re-ambulation via release from tail suspension (Recovery groups) with corresponding ambulatory controls (no HU)

Sample collection and processing

- Hearts are being collected from an ongoing HHC Tissue Sharing initiative
- After euthanasia, hearts are bisected into ventral (fixed) and dorsal (flash frozen) halves
- Collected samples are transported from UC Davis to NASA Ames Research Center for further processing and analysis

Assays

- RNAseq and qPCR analysis for differential expression of genes involved in cardiac disease, cardiac remodeling, stress responses, apoptosis, inflammation, macrophage activity, cell cycle and growth
- Oxidative damage assays (8-hydroxyguanosine, malondialdehyde, nitro-tyrosine)
- Histology and immunohistochemistry to assess macrophage infiltration, fibrosis and cardiomyocyte size

Questions being addressed by this study

- Which cardiac changes occur at the molecular level in response to the fluid shifts, musculoskeletal disuse and the relative inactivity that occur during long duration weightlessness?
- Are these changes reversible, and do the animal data suggest a significant risk to astronaut health?
- Are females as vulnerable as males to the adverse effects of long duration weightlessness?
- Does age affect the ability of cardiac tissue to adapt to and recover from long duration weightlessness?

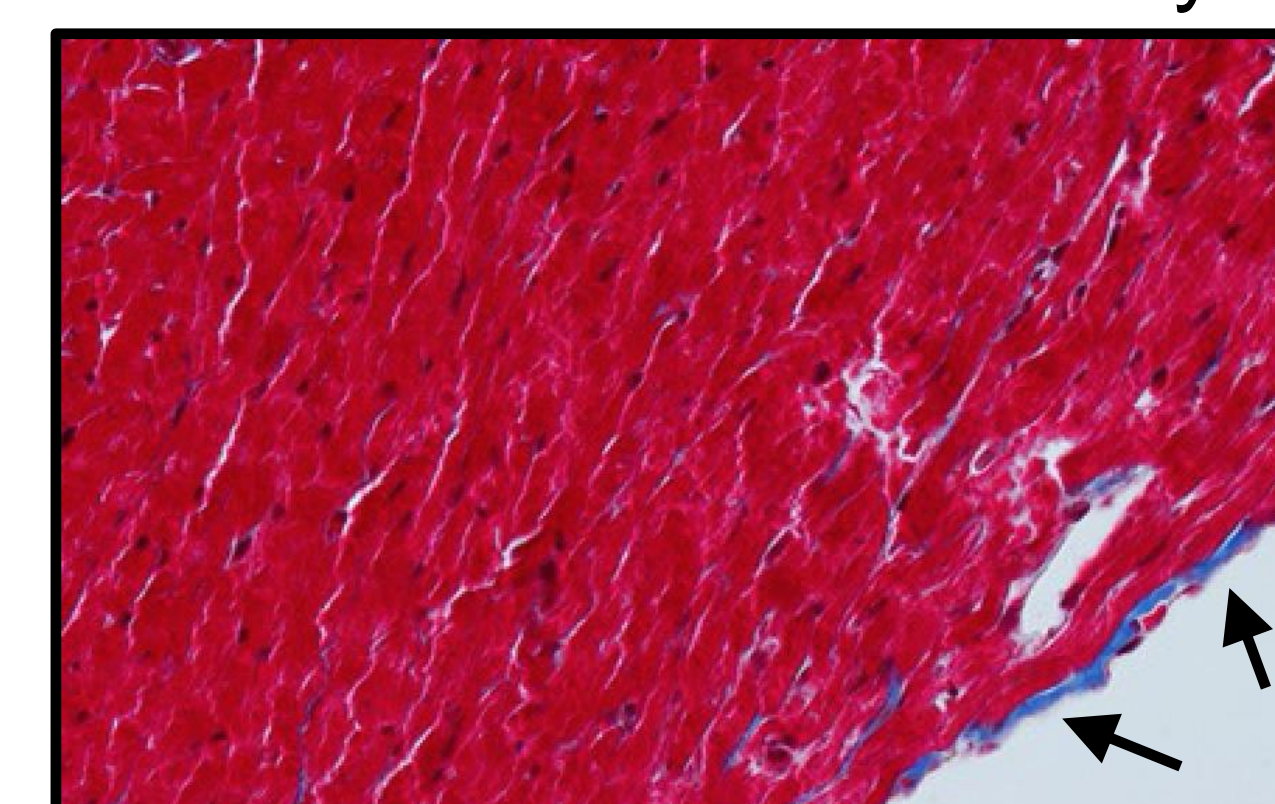
MILESTONES

Validation of RNA extraction protocol and assessment of RNA quality from frozen hearts received to date (completed)

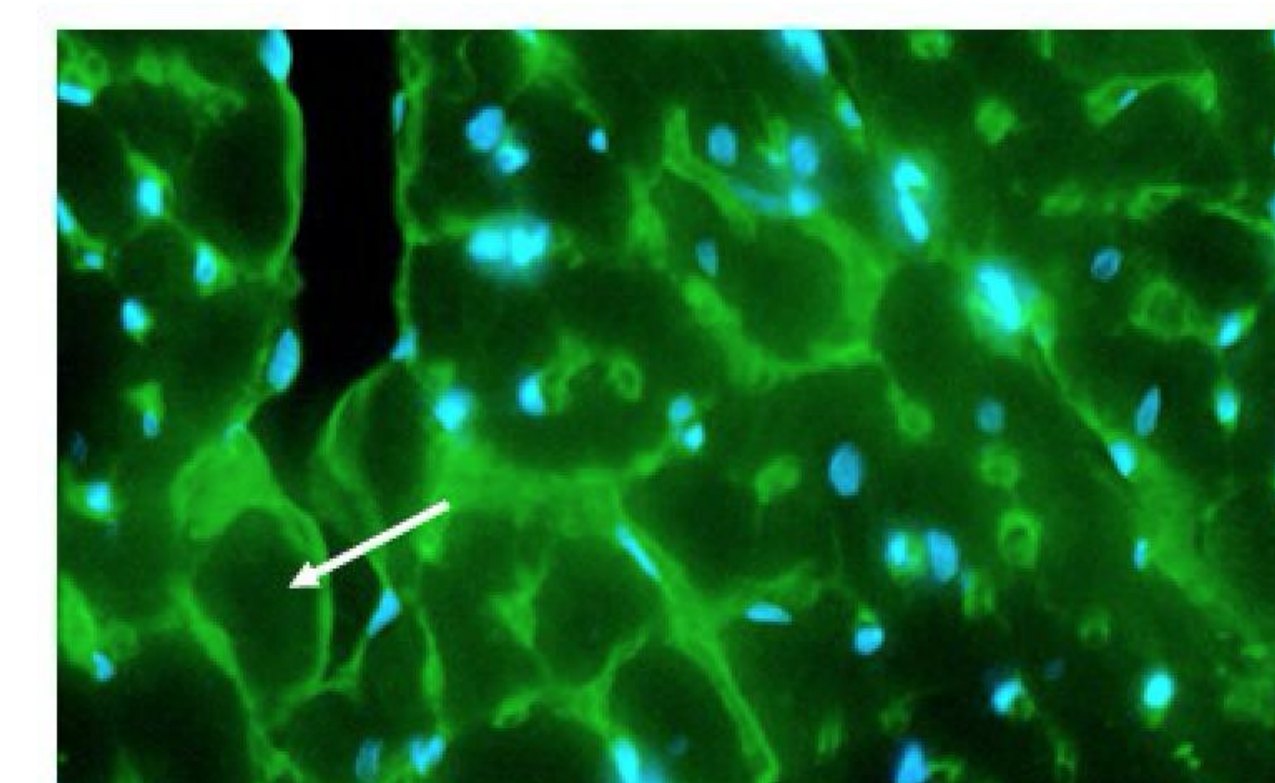
- RNA yield and concentration are optimal for all assays
- RNA quality is acceptable for RNAseq assays (RIN > 9 as assessed by Bioanalyzer)

Validation of heart tissue quality for immunohistochemistry applications (completed)

- Sample quality is satisfactory for immunohistological assessment of cardiomyocyte size and fibrosis



Mason Trichrome Staining to assess fibrosis in rat heart. Pericardium (black arrow) and fibrotic structures stain blue. Nuclei appear as dark punctate structures.



Measurement of cardiomyocyte size in rat heart. White arrows show a single cardiomyocyte with boundary delineated by immunoreactivity to anti-wheat germ agglutinin (WGA). Blue signals (DAPI stain) are from nuclei.

UPCOMING ACTIVITIES

- Immunohistological assessment of cardiomyocyte size, macrophage infiltration and fibrosis
- RNAseq
- Assessment of oxidative damage via measurement of 8-hydroxyguanosine immunoreactivity

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